Anim. Reprod., v.11, n.3, p.159-167, Jul./Sept. 2014

Effect of uterine environment on embryo production and fertility in cows

A.H. Souza[1,5], C.D. Narciso[2], E.O.S. Batista[1,3], P.D. Carvalho[4], M.C. Wiltbank[4]

[1]University of California, Cooperative Extension, Tulare, CA, USA.
[2]Veterinarian practitioner, Sequoia Veterinary Services, Tulare, CA, USA.
[3]Department of Animal Reproduction, FMVZ, University of São Paulo, SP, Brazil.
[4]Department of Dairy Science, University of Wisconsin-Madison, WI, USA.

Abstract

Oocyte fertilization rates in bovines following artificial insemination or natural mating are generally good (~90%). Curiously, only about one third of these pregnancies remain until 30 days post-AI in dairy cows. Thus, most pregnancies are lost between fertilization and early embryonic growth. Although classical pathways describing that lower progesterone post-AI is the main culprit to these early embryonic losses, a number of environmental factors such as heat-stress as well as novel concepts in bovine physiology including the effects of excessive negative energy balanced (NEB) and the insulin-resistant state experienced by high producing cows during the postpartum period can help explain the poor reproductive performance, generally observed in dairy herds world-wide. Thus, expanding the scientific knowledge in these critical areas in bovine fertility related to the evident impact of NEB and/or altered circulating and uterine metabolites in the postpartum period on oocyte quality, gamete transport, uterine environment, and early embryonic growth are of major importance to improve reproductive efficiency in modern high producing dairy cows.

Keywords: dairy cow, fertility, oocyte, uterus.

Introduction

Early embryo development and fertility of modern lactating dairy cows is far less than ideal, with only about 50% of the embryos been reported to be viable by 7 days after ovulation as compared to ~80% in non-lactating cows (Sartori *et al*., 2009). However, in the last couple of decades, we have gained important insights in understanding some of the complex interactions between the cow's environment, management, nutrition, level of milk production and possible breeding strategies that may improve overall fertility. For example, a growing body of evidences indicates that low circulating progesterone (P4) after ovulation in dairy cows seems to be related to conceptus growth and most likely mediated through changes in the endometrium rather than directly in the embryo (Clemente *et al*., 2009). However, several other blood parameters and management issues such as heat-stress may alter uterine environment, oocyte quality and embryo development. Thus, the aim of this manuscript is to review some factors associated with poor uterine health, embryo quality/development and fertility of dairy cows. It is important to highlight that multiple nutritional factors have been also associated with inadequate uterine environment, oocyte quality, and/or cow-immunity and health including diets that produce high levels of blood urea nitrogen (BUN), nutritional contaminants such as gossypol or mycotoxins, varying effects of different fat-sources as reviewed in Bisinotto *et al*. (2012). However, reviewing possible nutritional components that may influence fertility of dairy cows is out of the scope of the current manuscript. Emphasis will be given though to the detrimental impact of uterine infections or of excessive body weight losses that may occur in the postpartum period, with major direct effects in follicular dynamics, uterine tract physiology, embryo quality and ultimately conception results of dairy cows.

A model to study the impact of uterine environment of lactating cows

Trying to isolate areas in the whole body physiology and/or specific parts that might be responsible for poor fertility within the reproductive tract in bovines is a challenging task. For example, several research groups have described poor embryo quality and conception rate results in lactating cows, but it is unclear whether early embryonic growth up to day 7 post ovulation are reduced due to inadequate uterine environment and/or due to (i.e.) overexposure of oocytes to longer periods of high LH pulsatility - both hypothesis seem fairly plausible and could potentially be related to high milk production levels. Interestingly, it appears that embryo growth following day 7 post ovulation is compatible with high volumes of daily milk production since a growing body of scientific literature (Demetrio *et al*., 2007) supports the concept that the use of embryo transfer into lactating cows on day 7 seem to improve fertility in relation to regular AI. Further evidence to that is the fact that attempts to increase P4 levels after day 7 generally yield marginal to no results (Nascimento *et al*., 2013). These elements argue for at least nearly normal uterine environment to support pregnancy to term from day 7 to calving, and suggest

[5]Corresponding author: souza@ucdavis.edu
Phone: +1(559)684-3313; Fax+1(559)685-3319
Received: July 1, 2014
Accepted: July 18, 2014

that most issues with fertility of lactating cows is related to poor oocyte quality and/or inadequate uterine environment to support early embryo growth before day 7. Although recent findings argue for significant importance of some blood parameters (i.e. glucose) for embryo development after day 7 (Green *et al*., 2012). Despite of that, later findings indicate that oocyte quality can be improved in lactating cows through greater pre-ovulatory circulating P4 concentrations (Wiltbank *et al*., 2012), but little is actually known about the capacity of the uterus of lactating cows to cope with adequate fertilization and early embryo survival until day 7 after ovulation.

Although scarce, later publications were able to shed light on the events within the uterine tract from day 2 to day 7 after ovulation by utilizing laparoscopic transfer of IVF embryos into the oviduct (Rizos *et al*., 2010; Maillo *et al*., 2012). Both reports described drastic reductions in proportion of embryos that remained viable from day 2 to day 7 of the estrous cycle when these embryos were transferred into lactating cows as compared to nulliparous heifers (Rizos *et al*., 2010) or postpartum cows that were dried-off at calving (Maillo *et al*., 2012). The authors hypothesize that a combination of factors and complex associations may result in less than ideal endometrial environment in the lactating cow. Such factors are high milk production inducing low circulating P4 due to greater steroid metabolism, negative energy balance that lactating cows undergo at beginning of lactation, with remarkable increase in circulating non-esterified fatty acids (NEFA) as well as lower blood calcium, glucose and IGF-1.

Lower circulating P4 before and/or after ovulation has been extensively studied and associated with embryonic growth and conception results (Wiltbank *et al*., 2012); however, other blood parameters such as calcium and glucose concentrations have been largely overlooked and only recently studied more in depth to unravel their importance on dairy cow fertility. For example, low circulating calcium concentrations (<8.59 mg/dL) in the postpartum seem to be a key element to explain poorer immunity, uterine environment, and overall health in the lactating cow as described by Martinez *et al*. (2012). Additionally, lower circulating glucose and the resulting insulin-resistant state of postpartum cows to cope with high levels of milk production has only recently been associated with reduced embryonic growth (Green *et al*., 2012; Lucy *et al*., 2014). Thus, besides the classical findings on the importance of P4 levels post-ovulation on embryo development and maternal recognition, circulating calcium and glucose levels also appear to have central roles in providing an ideal uterine environment for the growing embryo and pregnancy. Further comprehensive studies looking into endometrial gene expression and histiotrophic milieu are urgently needed to bridge some of these missing concepts. Thus, later studies brought some important insights to help elucidate that the lactation status of the cow seem to be unarguably detrimental not only to oocyte quality as previously reported; but not surprisingly, milk production also appears to have a great impact in altering uterine environment making it less than ideal to the newly arrived embryo and even to maintain pregnancy to term.

Postpartum diseases and uterine environment in lactating cows

Cows experiencing clinical or subclinical uterine infections in the postpartum period have been reported by several research groups to have lower postpartum fertility (LeBlanc, 2008). Furthermore, greater milk production has been linked to lower circulating glucose and increased concentrations of other metabolites such as NEFA and BHBA in the postpartum period, which in turn has been linked to depressed migration activity and phagocytic/killing functions in polymorphonuclear (PMN) in the postpartum uterine lumen (Lucy *et al*., 2014). Nevertheless, most studies do not seem to indicate that milk production is a major drawback to uterine involution postpartum and/or subclinical uterine infections indicated by proportion of PMN cells. For example, in a recent study (Carvalho *et al*., 2013) in which we determined the number and percentage of PMN cells in endometrial smears taken nearly at 50 DIM, we found that level of milk production was largely unrelated ($P > 0.10$) to incidence of subclinical endometritis. These results corroborate with findings from a recent study (Scully *et al*., 2013), in which they found no significant differences in uterine diameter and fluid volume by ~50 DIM in cows that were lactating or dried-off just after calving time to try to isolate effects of lactation on uterine environment. Thus, milk production per se does not seem to be the major factor associated with the capacity of cows to undergo uterine involution postpartum. Surprisingly though, based on the results from Carvalho *et al*. (2013), it appears that greater proportions of PMN in uterine lumen have a direct effect in a number of embryo production parameters, as shown in Table 1 and Fig. 1. Thus, results from Carvalho *et al*. (2013) and Scully *et al*. (2013) provide compelling evidence that milk production has little effect on uterine health, but poor uterine environment (greater proportion of PMNs) can greatly impair oocyte fertilization capacity.

Obviously, the underlying physiology related to poor conception results in lactating cows lie in multiple factors including uterine infections that may disrupt normal postpartum follicular growth and delayed resumption of ovarian activity (anovulation), and/or direct effects in the uterine environment. It appears that the whole physiology of negative energy balance alongside with alterations in insulin signaling and IGF system, as well as deviations in circulating calcium in the early postpartum cow can have a major

impact in the process of normal immunity and uterine involution (Wathes *et al*., 2011; Martinez *et al*., 2012). For example, Wathes *et al*. (2011) randomized postpartum lactating cows to undergo mild or severe negative energy balance. Interestingly, these researchers described great upregulation patterns in several genes linked to metalloproteinase activity - an intrinsic part of uterine remodeling postpartum; indicating that uterine involution of cows under greater NEB seem to be altered and these deviations from normal physiology are likely caused by alterations in the IGF1/insulin signaling pathways in the endometrium of cows undergoing NEB. Thus, postpartum is characterized by a period of uterine tissue remodeling, and efforts to avoid excessive NEB and body loss experienced by cows during the first couple of weeks postpartum should improve uterine health and ultimately fertility.

Table 1. The effect of uterine polymorphonuclear cells (PMN) on ova/embryo recovery, fertilization, and transferable/freezable embryo numbers.

Endpoint	PMN <1% (n = 40)	PMN 1 to 5% (n = 13)	PMN >5% (n = 12)	P-value
CL number	17.7 ± 1.4	15.8 ± 2.3	17.2 ± 1.7	0.84
Total ova/embryos recovered	7.8 ± 1.1	9.2 ± 2.4	4.7 ± 1.1	0.10
% Recovery	41.5 ± 4.3^{ab}	55.5 ± 8.5^{a}	28.4 ± 6.3^{b}	0.04
Fertilized structures	5.9 ± 7.7^{a}	7.4 ± 1.9^{a}	2.3 ± 0.7^{b}	<0.01
% Fertilized structures	82.3 ± 3.4	81.8 ± 8.8	62.1 ± 11.4	0.14
Transferable embryos	4.6 ± 0.7^{a}	5.9 ± 1.7^{a}	1.8 ± 0.6^{b}	0.03
% Transferable/Total	62.3 ± 5.5	61.3 ± 11.6	52.0 ± 12.5	0.80
% Transferable/Fertilized	74.9 ± 5.4	71.3 ± 11.2	72.5 ± 12.7	0.95
Freezable embryos	4.4 ± 0.7^{a}	5.3 ± 1.6^{a}	1.8 ± 0.6^{b}	0.04
% Freezable/Total	59.4 ± 5.4	56.3 ± 12.2	48.9 ± 12.5	0.85
% Freezable/Fertilized	71.4 ± 5.3	65.4 ± 12.0	69.1 ± 13.3	0.99

^{a,b}Means with differing superscripts within same row are different P < 0.05. From Carvalho *et al*., 2013.

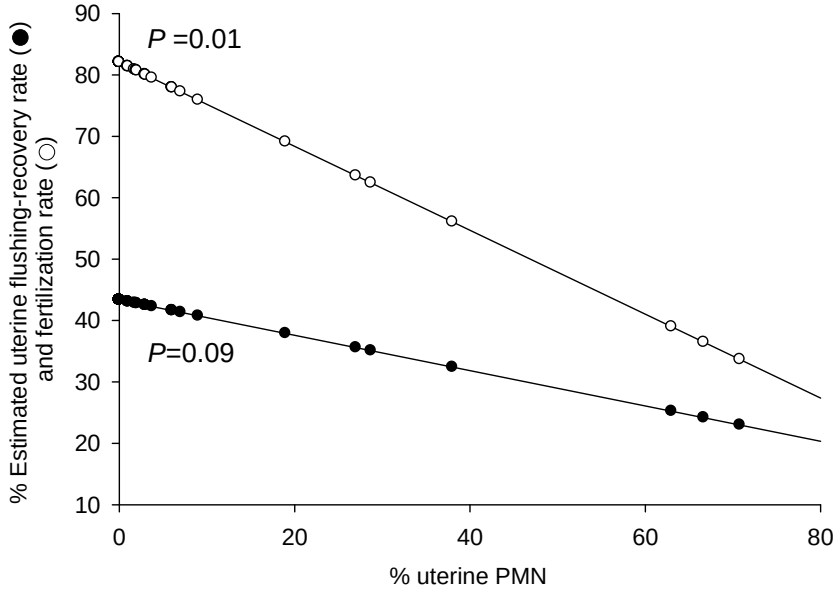

Figure 1. Estimated effect of uterine polymorphonuclear cell count (%PMN, endometrial swab) at the beginning of the superstimulation program of lactating Holstein cows on ova/embryo recovery rate (total structures collected divided by number of corpora lutea on the day of the collection) and fertilization rate (total number of fertilized structures divided by total structures collected). From Carvalho *et al*., 2013.

Impact of P4 and E2 before and near AI on uterine environment

Steroid hormones such as P4 and E2 have long been described to have major effects in the uterine tract of females, particularly in the endometrium (Bridges *et al*., 2013). For instance, Johnson *et al*. (1997) reported that uterine weights in ewes were greater near estrus compared to the mid-luteal phase, and that this increase in weight was due to estrogen-induced endometrial tissue hypertrophy rather than hyperplasia. This was consistent with data from other species, with regards to hormonal regulation of uterine morphology and function (Nayak and Brenner, 2002). Later studies also found great impact of steroid hormones in the transcriptome profile (Shimizu *et al*., 2010) and gene expression (Bridges *et al*., 2013) in the bovine endometrium, with adequate rise in E2 following pre-exposure to P4 being of major importance to prepare the uterus for embryo support and gestation as previously reviewed (Bridges *et al*., 2013). The low fertility of high producing cows may be partially explained by a suboptimal uterine environment likely as a result of lower circulating concentrations of both E2 during pro-estrus and P4 in the diestrus due to their greater steroid metabolism (Wiltbank *et al*., 2006). Interestingly, more precise synchronization systems are in part capable to counter act this excessive metabolism of steroids in the lactating cows and producing fairly satisfactory pregnancy results to timed AI breedings (Souza *et al*., 2008; Herlihy *et al*., 2012).

Thus, because of the dramatic changes induced by reproductive hormones in the uterine tract, we have hypothesized that ultrasonographic measurement of endometrial thickness (ET) near ovulation could be a good indicator of hormonal environment (i.e. low P4 and/or high E2), and could be used to assess whether the uterus has been exposed to adequate concentrations of hormones compatible with optimal fertility (Souza *et al*., 2011). In this study, there was a low to moderate correlation between circulating E2 and ET during this periovulatory period, but a number of our findings, combined with previous results (Jimenez-Krassel *et al*., 2009), provided strong support for the critical role of P4 in changes in ET. For example, it is clear that the major increase in ET occurred on the first day after PGF, concurrent with no detectable change in circulating E2 (Fig. 2). Further, the next 2 days had increases in serum E2 concentrations with no changes in ET. Nevertheless, the decrease in circulating E2 that followed GnRH treatment was accompanied by a decrease in ET, consistent with the critical role for E2, in the presence of low P4, to sustain a high ET. However, higher P4 concentrations were associated with much thinner ET, consistent with a critical underlying role for basal P4 in the periovulatory increase in ET.

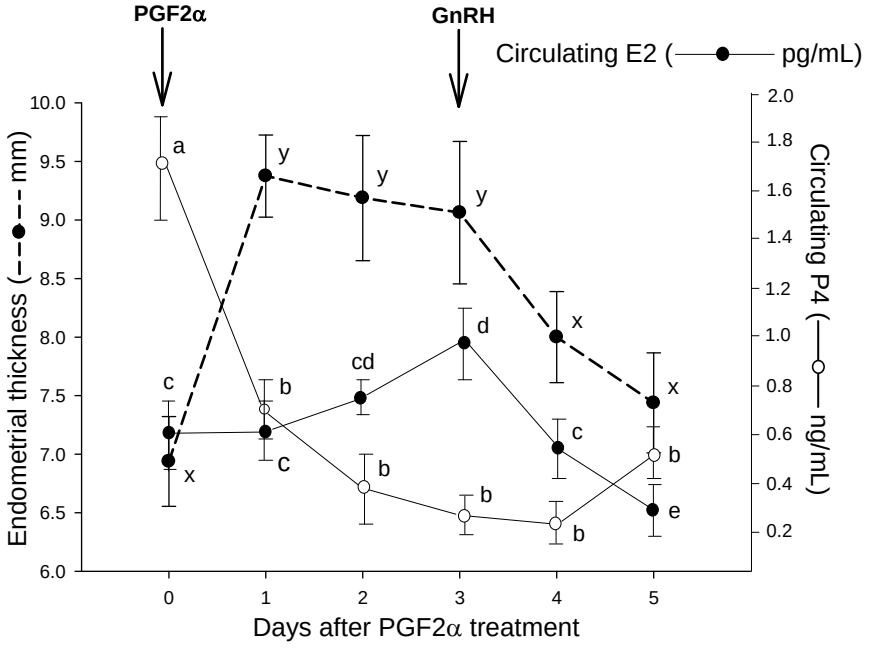

Figure 2. Profile of endometrial thickness (ET), and serum concentrations of P4 and E2 during the synchronized pro-estrus (from PGF to second GnRH treatment during the Ovsynch protocol) in lactating dairy cows (n = 8). Different letters throughout same line indicate statistical significance ($P < 0.05$). From Souza *et al*., 2011.

The profound effects of circulating E2 and P4 on the endometrium were also obvious in a larger dataset in that same publication - experiment 2 (Souza *et al*., 2011), in which we scanned lactating Holstein cows a single time near the time of the synchronized ovulation. It was clear that both presence of dominant follicles and lack of adequate luteolysis can alter even macroscopic features of the uterine morphology (Table 2, Fig. 3). Presumably, the lower conception results in cows deviating from expected values for ET around the time of ovulation, either due to low circulating E2 and/or high P4 near ovulatory period, indicate major impact of endocrine milieu on uterine environment.

We also observed marked changes in the ET in cows that were anovular before being enrolled in Ovsynch (Fig. 4). Surprisingly, out of the cows with smaller endometrial thickness (<8mm), most of them were not cycling (~60%) before been enrolled in Ovsynch for first postpartum AI. This was not true for cows with more adequate ET, in which only about 20 to 30% of them were anovular before Ovsynch. Thus, it appeared that P4 priming prior to the periovulatory period combined with a rapid decrease in circulating P4 after induction of luteolysis and greater circulating E2 near ovulation were critical for the remarkable changes in ET during the periovulatory period and ultimately conception results.

Table 2. Mean (± SEM) effects of presence or absence of at least one dominant ovarian follicle ≥10 mm in diameter or serum P4 class (low <0.5 ng/mL *vs*. high ≥0.5 ng/mL) in dairy cows 48 h after PGF treatment during an Ovsynch protocol - experiment 2.

End point	Dominant follicle (mm)		P-value	Serum P4 concentration (ng/mL)		P-value
	With	Without		Low	High	
No. cows	745	13		629	40	
Anovular (%)	8.6	15.4	0.38	8.8	7.5	0.78
Uterine tone[1]	2.8 ± 0.04	2.3 ± 0.26	0.02	2.8 ± 0.04	2.7 ± 0.17	0.52
ET[2] (mm)	9.9 ± 0.1	9.5 ± 1.0	0.41	10.0 ± 0.1	8.5 ± 0.3	< 0.01
ET ≤8 mm (%)	24.3	38.5	0.22	23.4	52.5	< 0.01
Estrus (%)	60.5	30.0	0.05	62.6	25.7	< 0.01
Ovulation (%)	94.9	7.7	< 0.01	92.7	87.5	0.05
P/AI d35-41 (%)	40.7	7.7	0.02	41.8	10.3	< 0.01
P/AI d58-64 (%)	37.1	7.7	0.02	37.7	10.3	< 0.01

[1]Uterine tone on scale of 1-4; [2]Endometrial thickness (ET). From Souza *et al*., 2011.

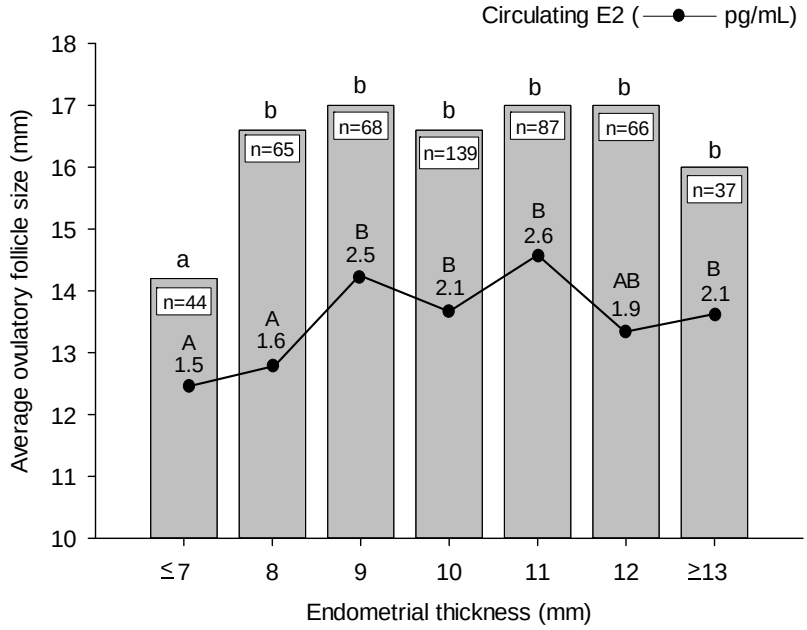

Figure 3. Average ovulatory follicle size and serum E2 concentrations for lactating dairy cows with varying endometrial thickness at 48 h after PGF treatment during the Ovsynch protocol. Cows with double ovulations not included in this analysis. [a,b]Means without a common superscript differed (P < 0.05).[A,B]Means without a common superscript tended to differ (P < 0.10). From Souza *et al*., 2011.

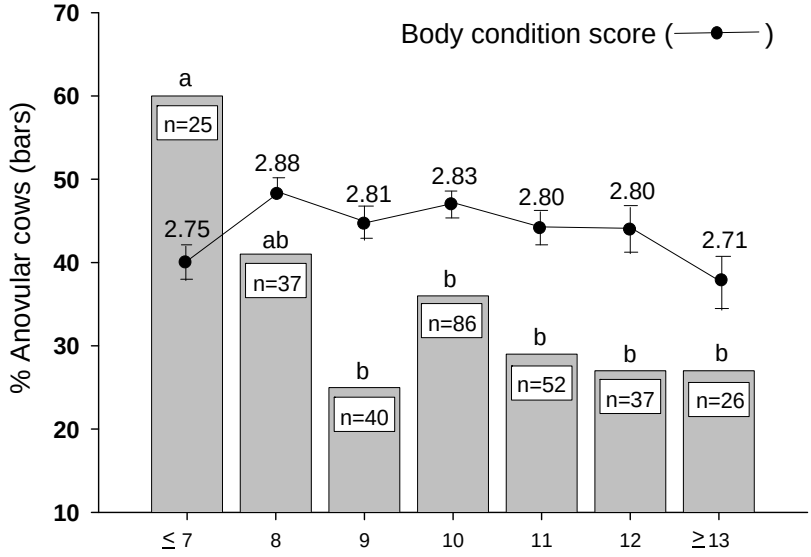

Figure 4. Relationship between cyclicity status before first postpartum timed AI, body condition score (BCS) and endometrial thickness (mm). [a,b]Means without a common superscript differed (P < 0.05). From Souza *et al*., 2011.

Effects of heat-stress on uterine environment

The effects of heat stress on oocyte and embryo quality in cattle (Al-Katanani *et al*., 2002; Ferreira *et al*., 2011; Silva *et al*., 2013) have been evaluated in several studies. However, reports exploring the effects of heat-stress on the uterine environment in cattle are rare. Apparently, heat-stressed cows have major alterations in the oviduct (Kobayashi *et al*., 2013) and uterine environment (Putney *et al*., 1988; Malayer and Woods, 1998) that in addition to perturbed follicle development and oocyte quality, may help explain the sharp drop in conception results experienced by herds during warmer months of the year. An early study (Putney *et al*., 1988), looked into PGF2α secretion in bovine endometrium tissues collected at day 17 of the estrous cycle from cyclic and pregnant cows and exposed to heat-stress conditions *in vitro*. Results from this trial are remarkable because heat-stress induced a rapid release of PGF2α regardless of reproductive status of the cow. More importantly, oxytocin failed to cause PGF2α release in endometrial tissues from pregnant cows kept at 39°C; however, when oxytocin was added to endometrial tissues at greater temperatures (42°C - to mimic heat stress conditions), it caused PGF2α release even in endometrium from pregnant cows. The authors suggest that the embryo may have more problems in inhibiting PGF2α release during heat stress. Besides that, other studies (Kobayashi *et al*., 2013) described changes in the oviduct after exposure to heat stress, with significant changes in oviduct-smooth muscle motility that could perturb transport of gametes in the oviduct and transport of the embryo to the uterus. This could actually explain lower fertilization rates found in dairy cows during summer months (Sartori *et al*., 2002). Additionally, it has been previously reported (Dreiling *et al*., 1991) that pregnant ewes exposed to warmer temperatures had much lower uterine blood flow (20 to 30% lower uterine flow for each 1°C increase in temperature), had greater circulating oxytocin and antidiuretic hormones, and produced offspring of much smaller size. Collectively, heat stress can induce important changes in the reproductive physiology of the pregnant uterus and in the future offspring, above and beyond its commonly discussed effects in oocyte quality and embryo development.

Body condition loss, negative energy balance (NEB) and their consequences on early embryo and fertility

At beginning of lactation, dry matter intake is generally low and not coupled with the large amounts of milk being produced. Thus, most modern dairy cows will undergo a normal period of NEB (Grummer, 2008), in which cows will utilize their own fat reserves to maintain high levels of milk production. As a result of fat mobilization, NEFA are released from adipose tissue into circulation (Weber *et al*., 2013). Plasma NEFA concentrations begin to increase even before parturition, reach peak concentrations near the time of parturition, and decrease in the postpartum period (Grummer *et al*., 2010). Increased postpartum NEFA concentrations are associated with a number of metabolic alterations including decreased milk production, ketosis, displaced abomasum, retained placenta, and metritis, all of which have been shown to alter uterine environment and oocyte quality (through its direct or indirect effects) with ultimate detrimental results in reproduction (Bisinotto *et al*., 2012). For example, cows with lower

body condition scores are far more likely to be anovular near the time of 1st AI and generally have much lower fertility and greater rates of embryonic losses (Santos and Rutigliano, 2009). Poor fertility of anovular cows can be attributed at least in part to early expression of oxytocin receptors in the uterine lumen and premature PGF2α release leading to short cycles (McCracken *et al*., 1999). Thus, uncoupled dry matter intake and level of milk production may lead to anovulation, which in turn will have a great impact in uterine lumen that will prematurely trigger CL regression, leading to embryonic death in cows having short-cycles.

Besides alterations in CL regression mechanisms through changes in oxytocin receptor expression induced by NEB, uterine involution may also be compromised by severe NEB with associated increased concentrations of NEFA commonly observed in the early postpartum in lactating dairy cows. As mentioned earlier, authors (Wathes *et al*., 2007, 2011) described that cows having severe NEB have much greater levels of inflammation in the uterus, and greater expression of genes involved in tissue remodeling. In this regard, we have recently shown (Carvalho *et al*., 2014) that levels of NEB and actual drop in body weight after calving are directly related to embryo quality, likely through major alterations in the uterine environment and oocyte quality. Body weight was monitored from calving to 70 ± 3 DIM, when cows were superovulated and flushed. In one of the analysis, we have divided cows in quartiles of body weight loss from calving to the 3rd week postpartum, in which Q1 included cows with least amount of body weight loss and Q4 cows with the greatest amount of body weight loss. All cows were superovulated with the same protocol and flushed at ~70 DIM. It seems clear that cows in Q4 (those that lost more weight from calving to 3rd week postpartum) had lower rates of fertilized structures, more degenerated embryos, and less transferable and freezable embryos (Fig. 5). These results are also in alignment with much worse conception rate for cows that lost more body condition score after freshening, as reported in the same manuscript from (Carvalho *et al*., 2014), highlighting the importance of minimizing NEB and excessive body score losses during the postpartum period in dairy cows.

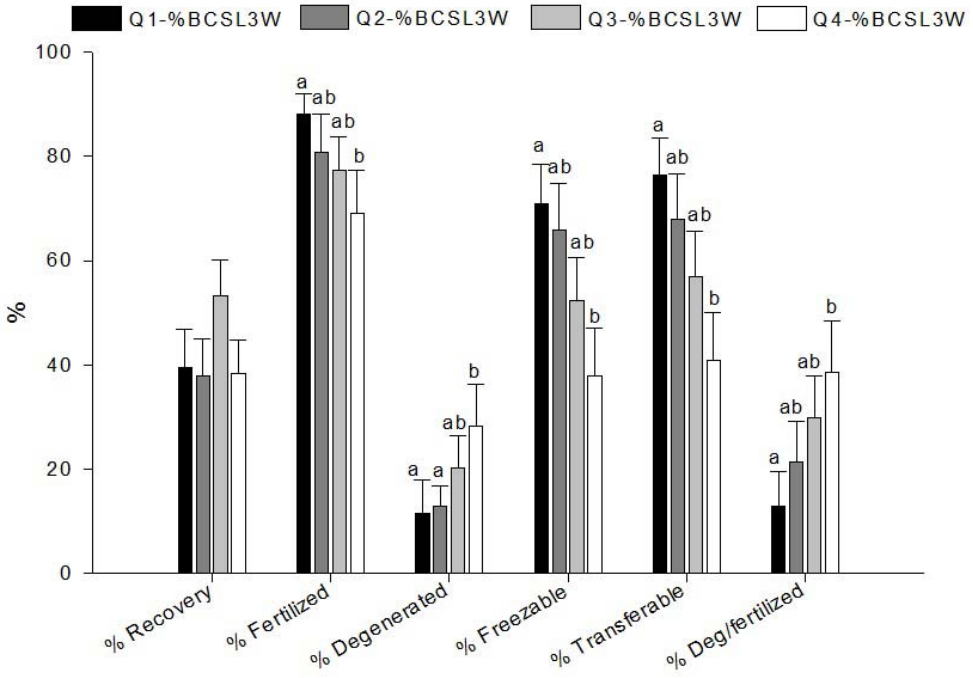

Figure 5. Embryo characteristics and percentage of body weight change from calving to 3 weeks postpartum in lactating Holstein cows. [a,b]Means without a common superscript differed (P < 0.05). Adapted from Carvalho *et al*., 2014.

Conclusions

A variety of factors related to management, calving process, and altered physiology of modern lactating cows seem to have important effects to orchestrate uterine environment, oocyte quality and ultimately fertility. Traditional theories that relate poor embryo development due to lower circulating P4 post-ovulation, although important, are obviously not the only culprit for poor embryo viability in modern cows. Some important alternative solutions to improve uterine environment in dairy cows may involve the utilization of better synchronization programs that create a more ideal profile of steroid hormones and follicle size near the time of AI, mitigate heat-stress in order to improve oocyte quality and uterine environment, and avoiding

excessive body weight losses in the fresh period that seem to be related to altered blood metabolites and poor embryo quality and fertility.

Grant support: Wisconsin Experiment Station and CAPES Brazil.

References

Al-Katanani Y, Paula-Lopes F, Hansen P. 2002. Effect of season and exposure to heat stress on oocyte competence in Holstein cows. *J Dairy Sci*, 85:390-396.

Bisinotto R, Greco L, Ribeiro E, Martinez N, Lima F, Staples C, Thatcher W, Santos J. 2012. Influences of nutrition and metabolism on fertility of dairy cows. *Anim Reprod*, 3:260-272.

Bridges G, Day M, Geary T, Cruppe L. 2013. Triennial Reproduction Symposium: deficiencies in the uterine environment and failure to support embryonic development. *J Anim Sci*, 91:3002-3013.

Carvalho P, Souza A, Sartori R, Hackbart K, Dresch A, Vieira L, Baruselli P, Guenther J, Fricke P, Shaver R. 2013. Effects of deep-horn AI on fertilization and embryo production in superovulated cows and heifers. *Theriogenology*, 80, 1074-1081.

Carvalho P, Souza A, Amundson M, Hackbart K, Fuenzalida M, Herlihy M, Ayres H, Dresch A, Vieira L, Guenther J. 2014. Relationships between fertility and postpartum changes in body condition and body weight in lactating dairy cows. *J Dairy Sci*, 97:3666-3683.

Clemente M, de La Fuente J, Fair T, Al Naib A, Gutierrez-Adan A, Roche J, Rizos D, Lonergan P. 2009. Progesterone and conceptus elongation in cattle: a direct effect on the embryo or an indirect effect via the endometrium? *Reproduction*, 138:507-517.

Demetrio D, Santos R, Demetrio C, Vasconcelos J. 2007. Factors affecting conception rates following artificial insemination or embryo transfer in lactating Holstein cows. *J Dairy Sci*, 90:5073-5082.

Dreiling CE, Carman III FS, Brown DE. 1991. Maternal endocrine and fetal metabolic responses to heat stress. *J Dairy Sci*, 74:312-327.

Ferreira R, Ayres H, Chiaratti M, Ferraz M, Araújo A, Rodrigues C, Watanabe Y, Vireque A, Joaquim D, Smith L. 2011. The low fertility of repeat-breeder cows during summer heat stress is related to a low oocyte competence to develop into blastocysts. *J Dairy Sci*, 94:2383-2392.

Green JC, Meyer JP, Williams AM, Newsom EM, Keisler DH, Lucy MC. 2012. Pregnancy development from day 28 to 42 of gestation in postpartum Holstein cows that were either milked (lactating) or not milked (not lactating) after calving. *Reproduction*, 143:699-711.

Grummer RR. 2008. Nutritional and management strategies for the prevention of fatty liver in dairy cattle. *Vet J*, 176:10-20.

Grummer RR, Wiltbank MC, Fricke PM, Watters RD, Silva-Del-Rio N. 2010. Management of dry and transition cows to improve energy balance and reproduction. *J Reprod Dev*, 56:S22-S28.

Herlihy MM, Giordano JO, Souza AH, Ayres H Ferreira RM, Keskin A, Nascimento AB, Guenther JN, Gaska JM, Kacuba SJ, Crowe MA, Butler ST, Wiltbank MC. 2012. Presynchronization with Double-Ovsynch improves fertility at first postpartum artificial insemination in lactating dairy cows. *J Dairy Sci*, 95:7003-7014.

Jimenez-Krassel F, Folger JK, Ireland JLH, Smith GW, Hou X, Davis JS, Lonergan P, Evans ACO, Ireland JJ. 2009. Evidence that high variation in ovarian reserves of healthy young adults has a negative impact on the corpus luteum and endometrium during estrous cycles in cattle. *Biol Reprod*, 80:1272-1281.

Johnson M, Redmer D, Reynolds L. 1997. Effects of ovarian steroids on uterine growth, morphology, and cell proliferation in ovariectomized, steroid-treated ewes. *Biol Reprod*, 57:588-596.

Kobayashi Y, Wakamiya K, Kohka M, Yamamoto Y, Okuda K. 2013. Summer heat stress affects prostaglandin synthesis in the bovine oviduct. *Reproduction*, 146:103-110.

LeBlanc S. 2008. Postpartum uterine disease and dairy herd reproductive performance: a review. *Vet J*, 176:102-114.

Lucy M, Butler S, Garverick H. 2014. Endocrine and metabolic mechanisms linking postpartum glucose with early embryonic and foetal development in dairy cows. *Animal*, 8:82-90.

Maillo V, Rizos D, Besenfelder U, Havlicek V, Kelly A, Garrett M, Lonergan P. 2012. Influence of lactation on metabolic characteristics and embryo development in postpartum Holstein dairy cows. *J Dairy Sci*, 95:3865-3876.

Malayer J, Woods V. 1998. Expression of estrogen receptor and maintenance of hormone-responsive phenotype in bovine fetal uterine cells. *Domest Anim Endocrinol*, 15:141-154.

Martinez N, Risco C, Lima F, Bisinotto R, Greco L, Ribeiro E, Maunsell F, Galvão K, Santos J. 2012. Evaluation of peripartal calcium status, energetic profile, and neutrophil function in dairy cows at low or high risk of developing uterine disease. *J Dairy Sci*, 95:7158-7172.

McCracken JA, Custer EE, Lamsa JC. 1999. Luteolysis: a neuroendocrine-mediated event. *Physiological Rev*, 79:263-323.

Nascimento A, Souza A, Guenther J, Dalla Costa F, Sartori R, Wiltbank M. 2013. Effects of treatment with human chorionic gonadotrophin or intravaginal progesterone-releasing device after AI on circulating progesterone concentrations in lactating dairy cows. *Reprod Fertil Dev*, 25:818-824.

Nayak NR, Brenner RM. 2002. Vascular proliferation and vascular endothelial growth factor expression in the rhesus macaque endometrium. *J Clin Endocrinol Metab*, 87:1845-1855.

Putney D, Malayer J, Gross T, Thatcher W, Hansen P, Drost M. 1988. Heat stress-induced alterations in the synthesis and secretion of proteins and prostaglandins by cultured bovine conceptuses and uterine endometrium. *Biol Reprod*, 39:717-728.

Rizos D, Carter F, Besenfelder U, Havlicek V, Lonergan P. 2010. Contribution of the female reproductive tract to low fertility in postpartum lactating dairy cows. *J Dairy Sci*, 93:1022-1029.

Santos J, Rutigliano H. 2009. Risk factors for resumption of postpartum estrous cycles and embryonic survival in lactating dairy cows. *Anim Reprod Sci*, 110:207-221.

Sartori R, Sartor-Bergfelt R, Mertens S, Guenther J, Parrish J, Wiltbank M. 2002. Fertilization and early embryonic development in heifers and lactating cows in summer and lactating and dry cows in winter. *J Dairy Sci*, 85:2803-2812.

Sartori R, Bastos MR, Wiltbank MC. 2009. Factors affecting fertilisation and early embryo quality in single- and superovulated dairy cattle. *Reprod Fertil Dev*, 22:151-158.

Scully S, Maillo V, Duffy P, Kelly A, Crowe M, Rizos D, Lonergan P. 2013. The effect of lactation on post-partum uterine involution in holstein dairy cows. *Reprod Domest Anim*, 48:888-892.

Shimizu T, Krebs S, Bauersachs S, BlumH, Wolf E, Miyamoto A. 2010. Actions and interactions of progesterone and estrogen on transcriptome profiles of the bovine endometrium. *Physiol Genomics*, 42:290-300.

Silva C, Sartorelli E, Castilho A, Satrapa R, Puelker R, Razza E, Ticianelli J, Eduardo H, Loureiro B, Barros C. 2013. Effects of heat stress on development, quality and survival of *Bos indicus* and *Bos taurus* embryos produced in vitro. *Theriogenology*, 79:351-357.

Souza AH, Ayres H, Ferreira RM, Wiltbank MC. 2008. A new presynchronization system (Double-Ovsynch) increases fertility at first postpartum timed AI in lactating dairy cows. *Theriogenology*, 70:208-215.

Souza AH, Silva E, Cunha A, Gümen A, Ayres H, Brusveen D, Guenther J, Wiltbank M. 2011. Ultrasonographic evaluation of endometrial thickness near timed AI as a predictor of fertility in high-producing dairy cows. *Theriogenology*, 75:722-733.

Wathes DC, Fenwick M, Cheng Z, Bourne N, Llewellyn S, Morris D, Kenny D, Murphy J, Fitzpatrick R. 2007. Influence of negative energy balance on cyclicity and fertility in the high producing dairy cow. *Theriogenology*, 68:S232-S241.

Wathes DC, Cheng Z, Fenwick MA, Fitzpatrick R, Patton J. 2011. Influence of energy balance on the somatotrophic axis and matrix metalloproteinase expression in the endometrium of the postpartum dairy cow. *Reproduction*, 141:269-281.

Weber C, Hametner C, Tuchscherer A, Losand B, Kanitz E, Otten W, Singh S, Bruckmaier R, Becker F, Kanitz W. 2013. Variation in fat mobilization during early lactation differently affects feed intake, body condition, and lipid and glucose metabolism in high-yielding dairy cows. *J Dairy Science*, 96:165-180.

Wiltbank MC, Lopez H, Sartori R, Sangsritavong S, Gümen A. 2006. Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. *Theriogenology*, 65:17-29.

Wiltbank MC, Souza AH, Carvalho PD, Bender RW, Nascimento AB. 2012. Improving fertility to timed artificial insemination by manipulation of circulating progesterone concentrations in lactating dairy cattle. *Reprod Fertil Dev*, 24:238-243.